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A new species of the calcareous sponge genus *Leuclathrina* (Calcarea: Calcinea: Clathrinida) from the Maldives

OLIVER VOIGT^{1,5}, BERNHARD RUTHENSTEINER², LAURA LEIVA¹,
BENEDETTA FRADUSCO¹ & GERT WÖRHEIDE^{1,3,4}

¹Department of Earth and Environmental Sciences, Palaeontology and Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner-Str. 10, 80333 München, Germany

²SNSB - Zoologische Staatssammlung München, Sektion Evertebrata varia, Münchhausenstr. 21, 81247 München, Germany

³GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner-Str. 10, 80333 München, Germany

⁴SNSB - Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Str. 10, 80333 München, Germany

⁵Corresponding author. E-mail: oliver.voigt@lmu.de, Tel.: +49 (0) 89 2180 6635; Fax: +49 (0) 89 2180 6601

Abstract

The diversity and phylogenetic relationships of calcareous sponges are still not completely understood. Recent integrative approaches combined analyses of DNA and morphological observations. Such studies resulted in severe taxonomic revisions within the subclass Calcinea and provided the foundation for a phylogenetically meaningful classification. However, several genera are missing from DNA phylogenies and their relationship to other Calcinea remain uncertain. One of these genera is *Leuclathrina* (family Leucaltidae). We here describe a new species from the Maldives, *Leuclathrina translucida* **sp. nov.**, which is only the second species of the genus. Like the type species *Leuclathrina asconoides*, the new species has a leuconoid aquiferous system and lacks a specialized choanoskeleton. Phylogenetic analyses of the partial 28S ribosomal RNA gene revealed that *L. translucida* **sp. nov.** is most closely related to a clade containing the exclusively asconoid genera *Ascandra*, *Levinella* and *Soleneiscus*, and to a clade of the likewise asconoid genus *Ernstia*. No close relationship exists to other members of the polyphyletic family Leucaltidae, or to any other leuconoid Calcinea. Our results suggest that the leuconoid aquiferous system of *Leuclathrina* evolved independently from that of other calcineans and that the family assignment of the genus has to be reconsidered. Because the latter requires a more comprehensive family level revision among many genera of Calcinea, we propose to formally retain the genus in Leucaltidae for the time being.

Key words: *Leuclathrina translucida* **sp. nov.**, leuconoid aquiferous system, Indian Ocean, phylogeny

Introduction

Calcareous sponges (class Calcarea, Bowerbank 1862) comprise about 740 valid species (Van Soest *et al.* 2017), and new species are frequently described. The current taxonomy of the class is still in conflict with DNA-based phylogenies, and many taxa below subclass-level are not monophyletic (Manuel *et al.* 2003; Manuel *et al.* 2004; Dohrmann *et al.* 2006; Voigt *et al.* 2012). Consequently, previously proposed scenarios on the evolution of important morphological traits, as e.g. the organisation of the aquiferous system (ideas proposed by Borojević *et al.* 1990, 2000, visualised by Manuel 2006) had to be rejected (Voigt *et al.* 2012). Although substantial progress towards a phylogenetic classification was recently achieved especially in the subclass Calcinea by application of integrative taxonomy (Córdoba-Luján & Klautau 2016; Klautau *et al.* 2013), some genera were not available for DNA-based studies and their phylogenetic affiliations remained uncertain. One of them is the genus *Leuclathrina* Borojević & Boury-Esnault, 1987. Until now, this genus was represented only by *Leuclathrina asconoides* Borojević & Boury-Esnault, 1987 from the Atlantic Ocean (Bay of Biscay). *Leuclathrina asconoides* has a leuconoid aquiferous system and the choanosome is free of skeletal elements, which is unique in Calcinea (Borojević & Boury-Esnault 1987). The genus is currently assigned to the family Leucaltidae Dendy and Row, 1913, which includes some other genera with a partially reduced choanoskeleton (Borojević & Boury-Esnault

1987). However, it also had been considered as ‘incertae sedis’ (Borojević *et al.* 1990). We here describe a new species of the genus from the Maldives that matches the morphological diagnosis of the genus, and study its phylogenetic relationships with the help of DNA-based phylogenies.

Material and methods

Collection and preparation for morphological observations. Specimens were collected in 2014, 2016 and 2017 at eight localities in the Maldives (Fig. 1). Fieldwork was conducted with permission of the Ministry of Fisheries and Agriculture, Malé, Republic of Maldives (sampling permits Nos. (OTHR)30-D/INDIV/2015/548, (OTHR)30-D/INDIV/2016/420, (OTHR)30-D/INDIV/2017/379; export permits Nos. (OTHR)30-D/INDIV/2015/603, (OTHR)30-D/INDIV/2016/702 and (OTHR)30-D/INDIV/2017/397). Sponges were fixed in 80 % ethanol. Parts of one specimens were fixed for histology in 4 % formaldehyde in seawater at 4°C overnight and subsequently transferred to 80 % ethanol in a dilution series. For spicule preparations, small pieces of tissue were dissolved in household-grade bleach containing 4 % sodium hypochlorite; the isolated spicules were washed five times with distilled water, spread on microscopic slides and mounted with Eukitt (Plano). To prepare histological sections with hard parts, tissues were stained in basic fuchsine, dehydrated in an ethanol dilution series (25 %, 50 %, 75 %, 99 %) and embedded in LRWhite resin for sectioning with a Leica 1600 saw microtome. 50–250 µm thick sections were prepared and stained on the surface with toluidine. For histological sections the formaldehyde-fixed specimen was gradually rehydrated, de-calcified in a 10 % EDTA-solution, dehydrated with an ethanol dilution series and embedded in paraffin wax. 5 µm sections were made with a Leica RM2255 microtome. The sections were dewaxed in xylene and stained using a Masson-Goldner trichrome staining kit (Morphisto). Stained sections were embedded with Eukitt (Plano).

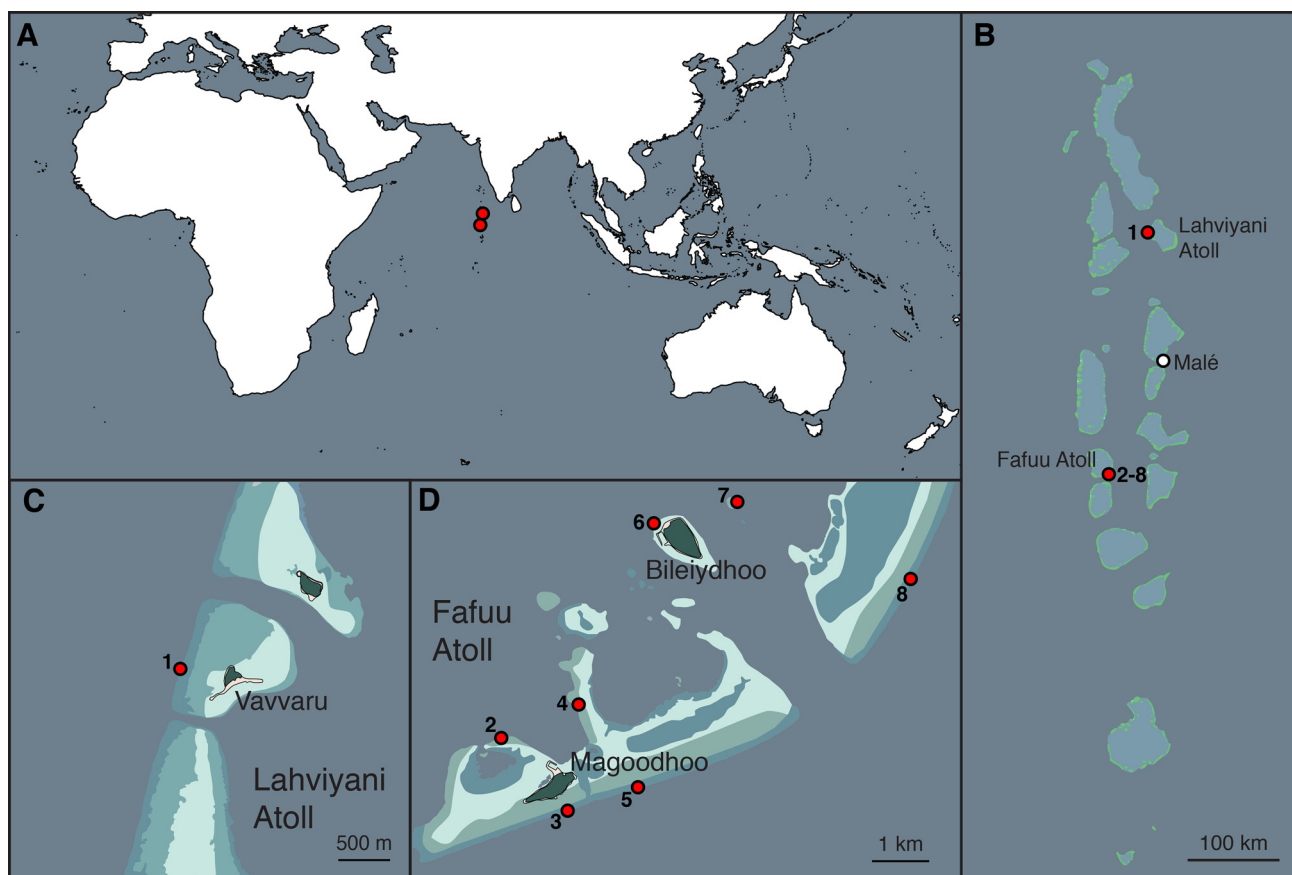


FIGURE 1. Sample localities (red dots) of *Leuclathrina translucida* sp. nov.: **A:** Location in the Indian Ocean (map generated with SimpleMapp, Shorthouse 2010); **B:** Location of the Lahviyani Atoll and the Fafuu Atoll in the Maldives; **C:** Detail of the type locality in the Lahviyani Atoll (1); **D:** Detail of the sample localities in the Fafuu Atoll. Dive sites: (2) Wallino, (3) Route 66 BIS, (4) Coral Garden, (5) Route 66, (6) Wall Street, (7) M&Ms, (8) Beyrufushi.

For μ CT analysis, dehydrated sponge tissue was incubated in 0.5 % iodine in ethanol for two days before scanning. μ CT scanning was performed with a Phoenix nanotom m at the Bavarian State Collection of Zoology at a voltage of 100 kV and a current of 60 μ A. 1440 projections were used to reconstruct a volume with a voxel size of 5.9 μ m. 3D visualization was generated by volume rendering with VGStudio MAX 3.0, using clipping planes. For better distinction of the soft tissue, volume-rendered images were post-processed with Photoshop CS6 (Adobe), in which parts of the image were highlighted by colouring. The specimens have been deposited at the Bavarian State Collection of Paleontology and Geology in Munich, Germany.

DNA extraction, PCR, sequencing. DNA of two specimens (SNSB-BSPG GW3934 and SNSB-BSPG GW4029) was extracted with the NucleoSpin Tissue kit (Macherey-Nagel); extracted DNA was quantified using a nanodrop. About 1 ng of genomic DNA was used as template in 25 μ l reaction-mixes to amplify the C-region of the 28S ribosomal RNA (rRNA) gene by PCR with the following primers: fwd: 5'-GAAAAGAAGCTTTGRARAGAGAGT-3' and rv: 5'-TCCGTGTTTCAAGACGGG-3' (Chombard *et al.* 1998); The thermoprofile of the PCR was: 95 °C: 1 min; 35x [95 °C: 30 s; 52 °C: 30 s; 72 °C: 30 s]; 72 °C: 1 min. Bi-directional DNA-sequencing of the PCR products was prepared with the BigDye Terminator sequencing kit v.3.1 (Applied Biosystems), and performed at the Sequencing Service at the LMU Biozentrum on an ABI 3730 capillary sequencer (Applied Biosystems). Forward and reverse sequences were assembled in Geneious R8 (<http://www.geneious.com>, Kearse *et al.* 2012) or CodonCode Aligner (www.codoncode.com) and checked by eye for correct base-calling. Sequences of the two specimens were submitted to the European Nucleotide Archive (available at: <https://www.ebi.ac.uk/ena/data/view/LT899664-LT899665>), and, along with morphological descriptions, to the sponge barcoding database (www.spongebarcoding.org; IDs: 1756, 1757).

TABLE 1. Taxonomy, voucher IDs, locality and accession numbers of species included in the phylogenetic analysis.

Species	Family	Voucher	Locality	Accession
' <i>Clathrina</i> ' <i>adusta</i>	Clathrinidae	QM G313665	Pacific, GBR	JQ272288
' <i>Clathrina</i> ' sp.	Clathrinidae	SNBS-BSPG.GW 975	Pacific, GBR	JQ272285
<i>Arturia sueziana</i>	Clathrinidae	SNSB-BSPG GW3120	Red Sea	KY366370
<i>Ascaltis reticulum</i>	Clathrinidae	UFRJPOR 6258	Mediterranean	HQ589014
<i>Ascandra contorta</i>	Clathrinidae	UFRJPor 6327	Mediterranean	HQ589011
<i>Ascandra corallicola</i>	Clathrinidae	UFRJPor 6329	Atlantic, Norway	HQ589012
<i>Ascandra</i> sp.	Clathrinidae	QM G323326	Tasmania	JQ272293
<i>Borojevia</i> (<i>Ascaltis</i>) sp.	Clathrinidae	QM G313824	Pacific, GBR	JQ272287
<i>Borojevia</i> aff. <i>aspina</i>	Clathrinidae	SMF11637	Red Sea	KY366400
<i>Borojevia aspina</i>	Clathrinidae	URFJPor 5495	Atlantic, Brazil	HQ589017
<i>Borojevia brasiliensis</i>	Clathrinidae	UFRJPor 5214	Atlantic, Brazil	HQ589015
<i>Borojevia cerebrum</i>	Clathrinidae	UFRJPor 6322	Mediterranean Sea	HQ589008
<i>Brattgardia</i> (<i>Guancha</i>) sp.	Clathrinidae	UFRJPor 6336	Atlantic, Norwegian Sea	HQ589019
<i>Brattgardia nansenii</i>	Clathrinidae	UFRJPor 6332	Atlantic, Greenland	HQ589013
<i>Clathrina antofagastensis</i>	Clathrinidae	MNRJ 9289	Chile	HQ589003
<i>Clathrina aurea</i>	Clathrinidae	MNRJ 8998	Atlantic, Brazil	HQ589005
<i>Clathrina blanca</i>	Clathrinidae	PMR-14307	Mediterranean	KC479081
<i>Clathrina</i> cf. <i>hondurensis</i>	Clathrinidae	PMR-14305	Mediterranean	KC479084
<i>Clathrina clathrus</i>	Clathrinidae	UFRJPOR 6315	Mediterranean	HQ589009
<i>Clathrina clathrus</i>	Clathrinidae	PMR-14308	Mediterranean	KC479083
<i>Clathrina conifera</i>	Clathrinidae	MNRJ 8991	Atlantic, Brazil	HQ589010
<i>Clathrina coriacea</i>	Clathrinidae	UFRJPor 6330	Atlantic, Norway	HQ589001
<i>Clathrina cylindractina</i>	Clathrinidae	UFRJPor 5206	Atlantic, Brazil	HQ589007
<i>Clathrina fjordica</i>	Clathrinidae	MNRJ 9964	Pacific, Chile	HQ589016

.....continued on the next page

TABLE 1. (Continued)

Species	Family	Voucher	Locality	Accession
<i>Clathrina helveola</i>	Clathrinidae	QM G313680	Pacific, GBR	JQ272291
<i>Clathrina lacunosa</i>	Clathrinidae	UFRJPor 6334	Atlantic, Norway	HQ589020
<i>Clathrina luteoculcitella</i>	Clathrinidae	QM G313684	Pacific, GBR	JQ272283
<i>Clathrina ramosa</i>	Clathrinidae	MNRJ 10313	Pacific, Chile	HQ589002
<i>Clathrina rotundata</i>	Clathrinidae	SMF11636	Red Sea	KY366399
<i>Clathrina rowi</i>	Clathrinidae	SMF11632	Red Sea	KY366394
<i>Clathrina rowi</i>	Clathrinidae	SMF11629	Red Sea	KY366390
<i>Clathrina rubra</i>	Clathrinidae	PMR-14306	Mediterranean	KC479082
<i>Clathrina sinusarabica</i>	Clathrinidae	SMF11631	Red Sea	KY366393
<i>Clathrina sinusarabica</i>	Clathrinidae	SNSB-BSPG GW3143	Red Sea	KY701522
<i>Clathrina</i> sp.	Clathrinidae	UFRJPor 5172	Atlantic, Brazil	HQ589004
<i>Clathrina</i> sp.	Clathrinidae	QM G316033	Pacific, GBR	JQ272284
<i>Clathrina wistariensis</i>	Clathrinidae	QM G313663	Pacific, GBR	JQ272303
<i>Ernstia (Clathrina)</i> sp.	Clathrinidae	UFRJPor 6107	Atlantic, Brazil	HQ589018
<i>Ernstia (Clathrina)</i> sp.	Clathrinidae	QM G313693	Pacific, GBR	AM180989
<i>Ernstia arabica</i>	Clathrinidae	SMF11627	Red Sea	KY366388
<i>Ernstia tetractina</i>	Clathrinidae	UFRJPor 5183	Atlantic, Brazil	HQ589021
<i>Soleneiscus hamatus</i>	Dendyidae	SNSB-BSPG GW2975	Red Sea	KY366365
<i>Soleneiscus radovani</i>	Dendyidae	QM G313661	Pacific, GBR	JQ272289
<i>Soleneiscus stolonifer</i>	Dendyidae	QM G313668	Pacific, GBR	JQ272290
<i>Leucaltis nodusgordii</i>	Leucaltidae	QM G316022	Pacific, GBR	JQ272302
<i>Leucettusa haeckeliana</i>	Leucaltidae	QM G323232	Tasmania	JQ272300
<i>Leucettusa imperfecta</i>	Leucaltidae	QM G323283	Tasmania	JQ272299
<i>Leucettusa imperfecta</i>	Leucaltidae	QM G323253	Tasmania,	JQ272301
<i>Leuclathrina translucida</i> sp. nov.	Leucaltidae	SNSB-BSPG GW3934	Indian Ocean, Maldives	LT899664
<i>Leuclathrina translucida</i> sp. nov.	Leucaltidae	SNSB-BSPG GW4029	Indian Ocean, Maldives	LT899665
<i>Leucascus</i> sp.	Leucascidae	QM G316051	Pacific, GBR	JQ272305
<i>Leucetta chagosensis</i>	Leucettidae	QM G316279	Pacific, Coral Sea	JQ272296
<i>Leucetta chagosensis</i>	Leucettidae	SNSB-BSPG GW3052	Red Sea	KY366368
<i>Leucetta chagosensis</i>	Leucettidae	SNSB-BSPG GW1122	Red Sea	KY366357
<i>Leucetta microraphis</i>	Leucettidae	QM G313659	Pacific, GBR	JQ272297
<i>Leucetta microraphis</i>	Leucettidae	SNSB-BSPG GW3196	Red Sea	KY775298
<i>Leucetta</i> sp.	Leucettidae	QM G313691	Pacific, GBR	JQ272298
<i>Leucetta villosa</i>	Leucettidae	QM G313662	Pacific, GBR	JQ272295
<i>Pericharax orientalis</i>	Leucettidae	QM G316295	Pacific, Coral Sea	JQ272294
<i>Levinella prolifera</i>	Levinellidae	QM G313818	Pacific, GBR,	JQ272292
<i>Lelapiella incrustans</i>	Lelapiellidae	QM G313914	Pacific, Vanuatu	JQ272306
<i>Murrayona phanolepis</i>	Murrayonidae	QM G313992	Pacific, Coral Sea	JQ272304

Alignment and phylogenetic analysis. The sequences of *L. translucida* **sp. nov.** were aligned to available sequences of Calcinea (see Table 1 for accession numbers and taxonomy), which represent the diversity of the subclass. Because both sequences of *L. translucida* **sp. nov.** were identical, only one was maintained in the dataset

for phylogenetic analyses. The alignment was refined with the help of the secondary structure of the 28S C-region rRNA (Voigt & Wörheide 2016); sites of uncertain homology were removed. The final dataset included 61 sequences and 396 sites. JModeltest (Posada 2008) was used to determine the best fitting model (TPM2uf+I+G), which was used in a Maximum Likelihood analysis with PhyML3 (Guindon *et al.* 2010), including a bootstrap analysis (1,000 replicates). Bayesian inference (BI) was performed with MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003) by running two independent chains for 5 million generations (with six substitution types and with rate parameters for invariable sites and rate heterogeneity). Every 200th tree was sampled, the first 25 % of sampled trees and parameters were omitted as burnin when summarizing the trees and parameters using the “sumt” and “sump” commands, respectively. Sufficient parameter sampling was verified by inspecting the parameter files with the software Tracer 1.6 (available at: <http://tree.bio.ed.ac.uk/software/tracer/>).

Results

Systematic description

Phylum Porifera Grant, 1836

Class Calcarea Bowerbank, 1862

Subclass Calcinea Bidder, 1898

Order Clathrinida Hartman, 1958

Family Leucaltidae Dendy & Row, 1913

Diagnosis. ‘Clathrinida with a tubular, ramified or even anastomosed cormus with many oscula, or individualised with a large central atrium and a single osculum. The sponge wall is composed of a distinct cortex sustained by a well-developed skeleton, and a choanosome. The skeleton of the choanosome and the atrial wall may be absent or composed of small and dispersed triactines and tetractines.’ (Borojević *et al.* 1990).

Remarks. The family Leucaltidae is not monophyletic (Voigt *et al.* 2012).

Genus *Leuclathrina* Borojević & Boury-Esnault, 1987

Diagnosis. ‘Leucaltidae with leuconoid organization, the skeleton of which is limited exclusively to the cortex. The choanosome is completely devoid of a skeleton.’ (Borojević & Boury-Esnault 1987).

Leuclathrina translucida sp. nov.

(Figs. 2 A–I, 3 A–F; Table 2)

Diagnosis. *Leuclathrina* with a yellow cormus composed of two distinct parts: an inhalant body part with macroscopically visible inhalant openings, and an exhalant body part, consisting of several transparent tubes, which emerge from the inhalant body part and occasionally reunite. The skeleton consists of equiradiate triactines lying tangentially in the sponge wall and forming a thick-layered skeleton in the inhalant body parts, and a much thinner layer in the wall of the exhalant tubes. The choanosome is free of spicules and restricted to the inhalant body part.

Type material. Holotype: SNSB-BSPG GW3934 (1 specimen), outer reef wall close to Vavvaru, Lhaviyani Atoll, Maldives (5.4203° N; 73.3508° E), coll. O. Voigt at 23 m on 9 Sept 2015; Paratypes: SNSB-BSPG GW4029 (1 specimen), outer reef wall close to Magoodhoo, Fafuu Atoll, Maldives (dive site 3.0748° N; 72.9663° E), coll. G. Wörheide at 20 m on 12 Oct 2016; SNSB-BSPG GW30279 (4 specimens) & SNSB-BSPG GW30280 (1 specimen), reef wall close to Magoodhoo, Fafuu Atoll, Maldives (dive site Wallino, 3.0870° N, 72.9558° E) coll. O. Voigt & B. Fradusco at 15–17 m on 29 Sep 2017; SNSB-BSPG GW30292 (1 specimen), inner reef close to Magoodhoo, Fafuu Atoll, Maldives (dive site Coral Garden, 3.09170° N, 72.96787° E), coll. O. Voigt & B.

Fradusco at 20 m on 29 Sep 2017; SNSB-BSPG GW30328 (1 specimen), outer reef, Fafuu Atoll, Maldives (dive site Beyrufushi, 3.11282° N, 73.02108° E), coll. O. Voigt & B. Fradusco at 17 m on 1 Oct 2017; SNSB-BSPG GW30350 (5 specimens), reef at Bileiydhoo, Fafuu Atoll, Maldives (dive site Wall Street, 3.12061° N, 72.97958° E), coll. O. Voigt & B. Fradusco at 17 m on 2 Oct 2017; SNSB-BSPG GW30361 (1 specimen), reef between Magoodhoo and Bileiydhoo, Fafuu Atoll, Maldives (dive site M&Ms, 3.12429° N, 72.99201° E), coll. O. Voigt & B. Fradusco at 15 m on 2 Oct 2017; SNSB-BSPG GW30377 (3 specimens), reef wall close to Magoodhoo, Fafuu Atoll, Maldives (dive site Wallino, 3.0870° N, 72.9558° E) coll. O. Voigt, B. Fradusco & G. Wörheide at 20 m on 4 Oct 2017; SNSB-BSPG GW30419 (2 specimens), outer reef, E of Magoodhoo, Fafuu Atoll, Maldives (dive site Route 66, 3.07902° N, 72.97797° E), coll. O. Voigt & B. Fradusco at 15 m on 2 Oct 2017.

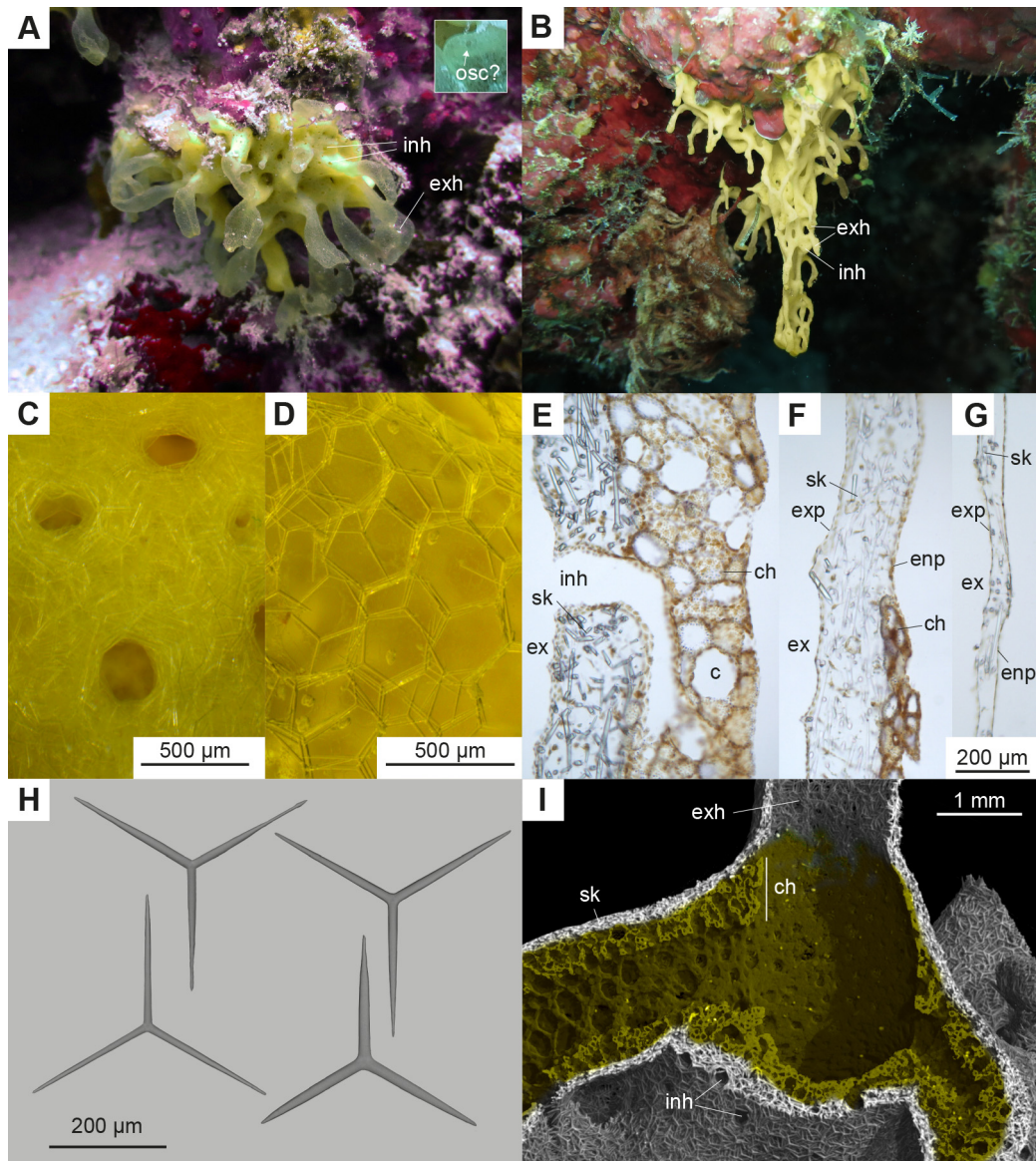


FIGURE 2. *Leuclathrina translucida* sp. nov. **A:** Typical growth form in situ (holotype). The inset shows an opening at the end of an exhalant tube of another specimen, possibly an osculum; **B:** Specimen (SNSB-BSPG 30280) with a hanging growth form and smaller exhalant tubes. **C:** Detail of the outer sponge wall of the inhalant region with inhalant openings; **D:** Detail of the outer sponge wall of the exhalant tubes, showing the hexagonally arranged triactines around the translucent tissue areas (C-D: material fixed in 80 % ethanol); **E-G:** Section through the sponge wall at different regions of the sponge body; **E:** In the inhalant region the skeleton consists of several layers of tangential triactines, the choanosome is free of spicules; **F:** Transition between the inhalant and exhalant region: the choanosome gets thinner and is replaced by a layer of endopinacoderm. **G:** The thickness of the sponge wall is strongly reduced in the more distal parts of the exhalant tubes. **H:** Spicules (triactines); **I:** 3D reconstruction of the skeleton and the choanosome (coloured). **Abbreviations:** c= canal (inhalant or exhalant); ch= choanosome; enp= endopinacoderm; ex= exterior of the sponge wall; exh= exhalant body part; exp= exopinacoderm; inh= inhalant body part or inhalant opening; sk= skeleton.

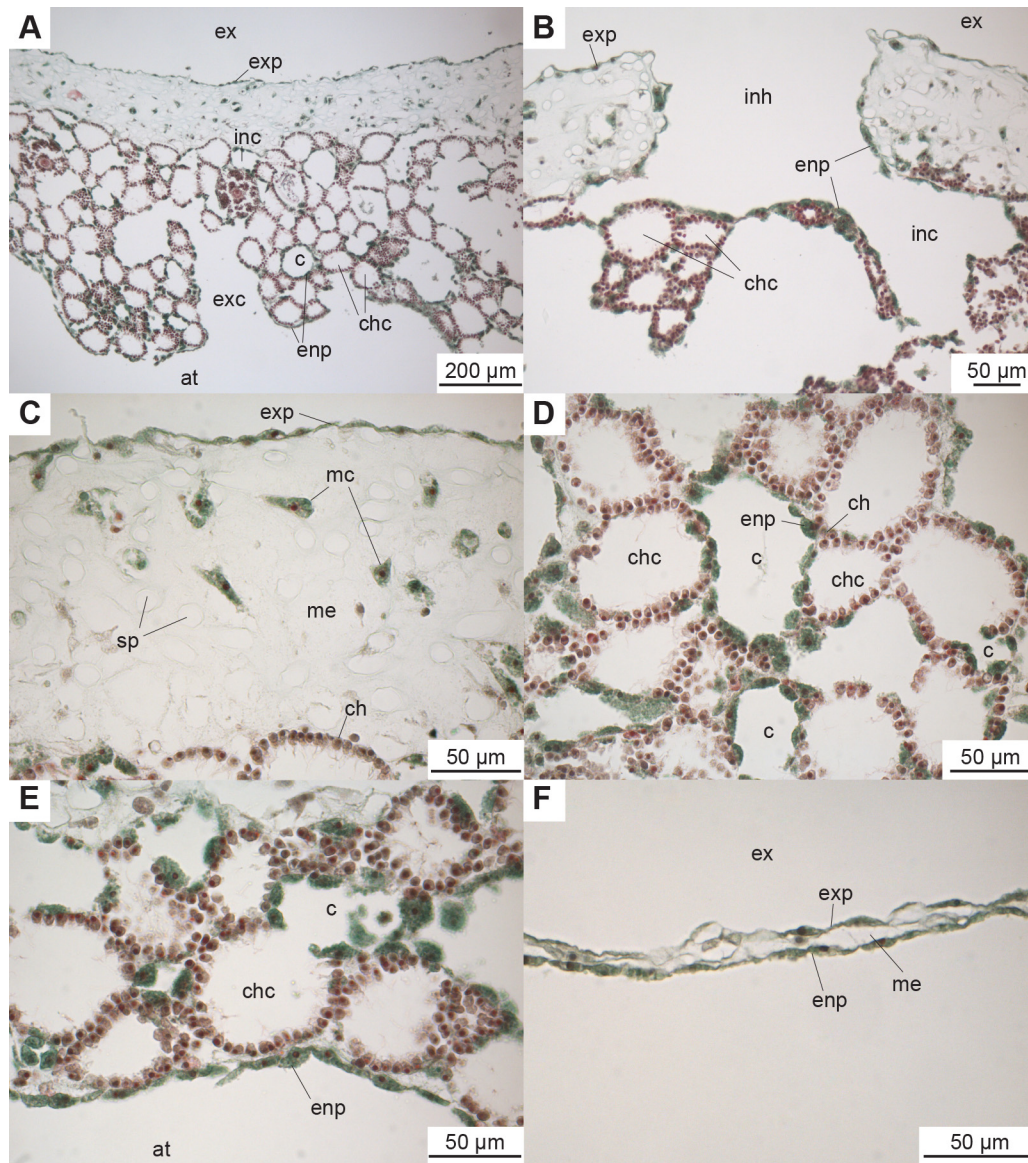


FIGURE 3. Histology of *L. translucida* sp. nov. (SNSB-BSPG 30279), decalcified: **A:** Section through the sponge wall including the outer skeletal layer, the choanosome and the atrial cavity; **B:** Detail of an inhalant opening leading to an inhalant canal; **C:** Detail of the outer skeletal layer, which is delimited by exopinacocytes on the external surface; only few cells occur in the mesohyl of this layer; **D:** Detail of the choanosome, with spherical choanocyte chambers and canals of the aquiferous system (inhalant or exhalant), the latter is lined by endopinacocytes. **E:** Choanoderm adjacent to the atrial cavity. The wall of the atrial cavity is lined by endopinacocytes. **F:** Section through the wall of an exhalant tube, consisting of an external layer of exopinacocytes and an internal layer of endopinacocytes, and a thin mesohyl layer in between (originally with a few spicules). **Abbreviations:** at: atrial cavity; c: canal (inhalant or exhalant); ch: choanocyte; chc: choanocyte chamber; enp: endopinacoderm; ex: external side of the sponge; exc: exhalant canal; exp: exopinacoderm; inc: inhalant canal; inh: inhalant opening; mc: mesohyl cell; me= mesohyl; sp: dissolved spicule.

Type locality. Vavvaru, Lhaviyani Atoll, Maldives

Etymology. Named for its translucent exhalant tubes.

Colour. Yellow in life (Figs. 2 A, B) and in 80 % ethanol.

Growth form. Two different growth forms were observed. The majority of sponges have a cushion-shaped cormus, which is attached to the substrate with its base (Fig. 2 A). The holotype measures 3.5 cm x 2.3 cm (fixed as two fragments). Several translucent exhalant tubes (about 2 -2.7 mm wide in the holotype) emerge from the base and sometimes reconnect at their upper parts (Fig. 2 A). Only on few occasions, small openings were visible in the exhalant tubes of living specimens, which are most likely oscula (Fig. 2 A, inset). In fixed material, the oscula are not visible, either because of contraction or due to damage of the delicate exhalant tubes. Two specimens showed a

modified growth form and were hanging from ceilings of small or larger overhangs (Fig. 2 B). In the larger specimen of this growth form the inhalant body parts were forming a mesh-like structure in some parts of the cormus (Fig. 2 B). Exhalant tubes were less developed or contracted in comparison to the more typical growth form (Fig. 2 B). In both growth forms, the inhalant body parts have inhalant openings of about 150 to 270 μm in diameter (Figs. 2 A, C), which are lacking in the exhalant tubes (Fig. 2 D).

Skeleton. The body wall of the inhalant part of the sponge and at the transition between the inhalant and exhalant body parts is supported by a thick layer of tangential triactines (Figs. 2 E, F). Towards the more distal parts of the exhalant tubes, the skeleton is getting much thinner (Fig. 2 G), consisting of only a thin layer of triactines (Figs. 2 C, G). In this region the triactines leave approximately hexagonal shaped areas that are free of spicules, resulting in the transparent appearance of the exhalant tubes (Fig. 2 D).

Spicules. Equiangular, equiradial triactines (Fig. 2 H). The actines are straight and conical, with sharp or blunt tips. In some triactines, the actines are locally slightly thickened near the tip (Fig. 2 H). Spicule sizes of the holotype are: inhalant region: actine length: 147–194.4–247 μm , actine width: 12–14.4–18 μm ; exhalant region: actine length: 143–212.1–256 μm , actine width: 9–14.2–19 μm . Spicules measurements of three paratypes are provided in Table 2.

TABLE 2. Spicule dimensions of *L. translucida* sp. nov.; ‘SD’= standard deviation, ‘n’ depicts the number of measured spicules.

Specimen (body part)	Actine length in μm				Actine width in μm				n
	Min	Mean	SD	Max	Min	Mean	SD	Max	
SNSB-BSPG GW3934 (inhalant)	147	194.4	23.4	247	12	14.4	1.3	18	30
SNSB-BSPG GW3934 (exhalant)	143	212.1	24.9	256	9	14.2	2.3	19	30
SNSB-BSPG GW4029 (inhalant)	44	183.9	35.0	222	7	15.2	2.5	20	30
SNSB-BSPG GW4029 (exhalant)	68	193.0	23.1	217	8	14.3	2.3	21	30
SNSB-BSPG GW30279 (inhalant)	118	180.9	21.9	223	9	13.8	1.9	17	30
SNSB-BSPG GW30279 (exhalant)	144	190.3	19.7	220	8	11.7	1.9	16	30
SNSB-BSPG GW30377 (inhalant)	154	187.2	17.8	213	10	13.3	1.6	16	30
SNSB-BSPG GW30377 (exhalant)	143	188.5	20.0	222	8	12.1	2.5	17	30

Histology and organisation of the aquiferous system. The sponge wall of the inhalant body part consists of a thick skeletal layer and the spicule-free choanosome (Figs. 2 E, I, 3 A). The skeletal layer is delimited by an exopinacoderm on the outer side and is perforated by inhalant openings, which are lined by endopinacocytes and lead to narrower inhalant canals (Figs. 2 E, 3 B). The skeletal layer contains only few mesohyl cells (Fig. 3 C). The choanosome layer comprises spherical choanocyte chambers, between which inhalant and exhalant canals occur, which are lined by endopinacocytes (Fig. 3 D). The exhalant canals unite and increase their diameter until they open into the central atrium (Fig. 3 A). The 3D reconstruction reveals that the diameter of the openings to the atrium increases with the thickness of the choanosome (Fig. 2 I). A layer of endopinacocytes delimits the wall of the central atrial cavity (Fig. 3 E). Towards the transition of the inhalant to the exhalant tubes the choanoderm decreases its width until it disappears, while the thickness of the skeletal layer remains the same in this region (Figs. 2 F, 3 I). The latter however decreases in the more distal parts of the exhalant tubes, until it only contains a thin layer of spicules within a thin mesohyl (Fig. 2 G, Fig. 3 F). Oscula were not visible in the fixed material.

Ecology and distribution. The specimens were growing on hard substrate, in most cases on vertical parts of reef walls, on living or dead corals, or hanging from ceilings of small overhangs, in depths between 15 and 23 m. The samples in this study originated from the Maldives; a wider distribution in the Indo-Pacific is mentioned by Coleman (2001), but not further specified.

Remarks. Photographs of *Leuclathrina translucida* sp. nov. have been published previously, e.g. as ‘*Clathrina* sp.’ in the field guide ‘Marine Life of the Maldives’ (Coleman 2001, page 36).

Leuclathrina translucida sp. nov. differs from the type species of the genus, *L. asconoides* in several aspects and can easily be distinguished. *Leuclathrina asconoides* is smaller (3.5 mm) and has a globular shape; its exhalant tubes (‘chimneys’) are shorter (a few millimetres, Borojević & Boury-Esnault 1987). Triactines of *L. asconoides* can grow to a larger size compared to *L. translucida* sp. nov.: The length of the actines varies between 156–598 µm, and their width between 13–36 µm (Borojević & Boury-Esnault 1987).

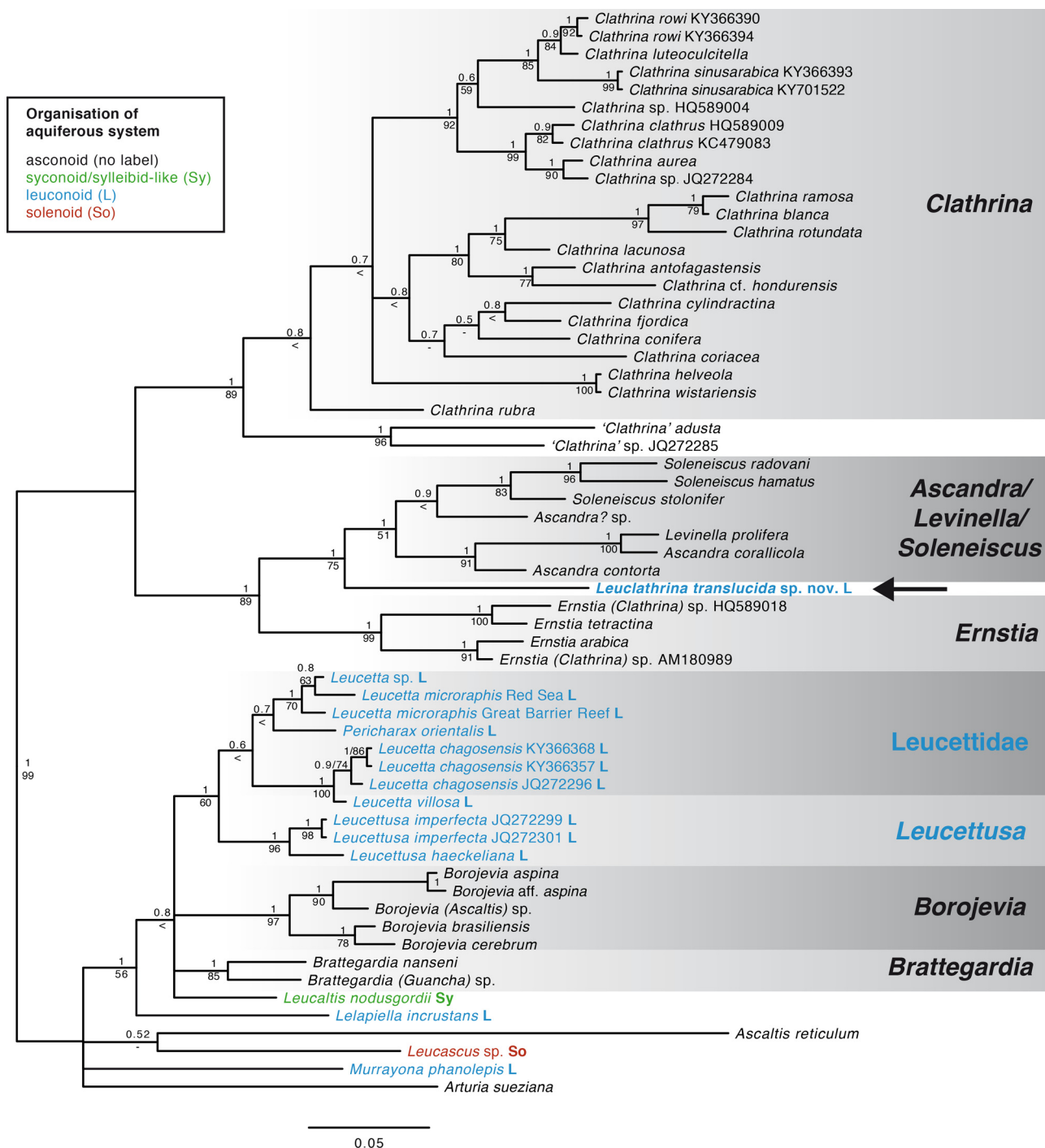


FIGURE 4. Phylogenetic position (arrow) of *Leuclathrina translucida* sp. nov. within Calcinea (midpoint rooted Bayesian phylogeny). Posterior probability (BI) and bootstrap values of the Maximum Likelihood (ML) analysis are given at the nodes, respectively (‘-’= node not present in ML tree, ‘<’= bootstrap values below 50). For undetermined species that probably belong to more recently established genera, the original genus affiliation is provided in brackets, ‘?’ marks a sample of uncertain identification. The organisation of the aquiferous system of the species is provided (see legend in the inset).

Phylogenetic position of *Leuclathrina translucida* sp. nov.

According to our phylogeny, *L. translucida* sp. nov. is closely related to a clade comprising *Ascandra* Haeckel, 1872, *Levinella* Borojević & Boury-Esnault, 1986 and *Soleneiscus* Borojević et al., 2002, and to a clade of species of *Ernstia* Klautau et al., 2013 (Fig. 4). All these taxa together form a sister group to a clade of *Clathrina* sensu Klautau, 2013 (species without tetractines) and two tetractine-bearing ‘*Clathrina*’ specimens (‘*Clathrina*’ *adusta* Wörheide & Hooper, 1999 and an undetermined species). With the only exception of *Leuclathrina translucida* sp. nov., these sponges are all asconoid. Sponges with more complex aquiferous systems (*Murrayona* Kirkpatrick, 1910, *Pericharax* Poléjaeff, 1883, *Lelapiella* Vacelet, 1977, *Leucettusa* Haeckel, 1872, *Leucetta* Haeckel 1972, and *Leucaesus* Dendy, 1893) occur in the sister clade of the aforementioned taxa. This sisterclade also includes additional asconoid genera: *Ascaltis* Haeckel, 1872, *Borojevia* Klautau et al., 2013 and *Brattgardia* Klautau et al., 2013. Because this part of the phylogeny is not resolved (polytomies in the Bayesian phylogeny) and/or the support for the relationships of most genera is low, it is not further treated.

Discussion

Leuclathrina translucida sp. nov. is closely related to the *Ascandra/Levinella/Soleneiscus* clade and to *Ernstia*. Morphologically, our new species can be unambiguously diagnosed as *Leuclathrina*. Because of the distinct morphological organization of *Leuclathrina translucida* sp. nov., especially regarding the aquiferous system and the cornus, this species cannot be included in any of the closest related genera *Ascandra/Levinella/Soleneiscus*, or *Ernstia*. However, since no molecular data of the type species *L. asconoides* is available yet, the monophyly of the genus *Leuclathrina* needs to be tested by means of molecular analyses, especially when considering the many problems that have been observed in other genera (Voigt et al. 2012).

It was speculated that the leuconoid organisation of *Leuclathrina* differed from that of *Leucettusa* and *Leucaltis* Haeckel, 1872, because in both these genera the aquiferous system has some remnants of a radial organization (Borojević et al. 1990). In concordance with this idea, the phylogenetic position of *L. translucida* sp. nov. within a clade of exclusively asconoid calcineans suggests that this species has developed a leuconoid aquiferous system independently from other leuconoid sponges in Calcinea. It is therefore likely that the choanoskeleton is not reduced, but primarily absent in *Leuclathrina*. Furthermore, *L. translucida* sp. nov. is not closely related to the genera *Leucaltis* or *Leucettusa*, which belong to the family Leucaltidae. The family Leucaltidae is polyphyletic (Voigt et al. 2012), and our results suggest that a transfer of *Leuclathrina* to another family will be required. However, due to the recent revisions and introduction of new genera in Calcinea (Córdoba-Luján & Klautau 2016; Klautau et al. 2013), the family designation for *Ascandra* (Leucaltidae), *Soleneiscus* (Dendyidae De Laubenfels, 1936), *Levinella* (Levinellidae Borojević & Boury-Esnault, 1986) and *Ernstia* (Clathrinidae Minchin, 1900) has to be reconsidered. Only to avoid additional taxonomic confusion, we decided to formally retain *Leuclathrina* in Leucaltidae until a more general revision of the family-level taxonomy of Calcinea will be available.

Author contributions

Fieldwork: Gert Wörheide, Benedetta Fradusco, Oliver Voigt

Molecular laboratory work: Laura Leiva

μCT analysis: Bernhard Ruthensteiner, Oliver Voigt

Morphological observations, taxonomy, phylogenetic analysis and drafting of manuscript: Oliver Voigt

Revision of the manuscript: All authors

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